Received: 15 February 2023 | Revised: 7 June 2023 | Accepted: 7 June 2023

DOI: 10.1002/btpr.3372

RESEARCH ARTICLE

Cell Culture and Tissue Engineering

BIOTECHNOLOGY PROGRESS

Characterization of lipoprotein supplement and influence of its oxidized lipid content on cell culture performance and monoclonal antibody production by a SP2/0 hybridoma cell line

Rémy Moisant[1] | Elliott Cowles[1] | Léonie Broutel[1] | Véronique Deparis[1] | Anthony Baud[1] | Joerg von Hagen[2] | Harald Kolmar[3]

[1]Manufacturing Science and Technology, Merck Serono SA (an affiliate of Merck KGaA, Darmstadt, Germany), Corsier-sur-Vevey, Switzerland

[2]Geschäftsführer ryon – GreenTech Accelerator Gernsheim GmbH, Gernsheim, Germany

[3]Applied Biochemistry Department, Technical University of Darmstadt, Darmstadt, Germany

Correspondence
Rémy Moisant, Manufacturing Science and Technology, Merck Serono SA (an affiliate of Merck KGaA, Darmstadt, Germany), Route de Fenil Z.I.B, 1804 Corsier-sur-Vevey, VD, Switzerland.
Email: remy.moisant@merckgroup.com

Funding information
Merck Serono SA (an affiliate of Merck KGaA, Darmstadt, Germany)

Abstract

A challenging aspect with the use of the Sp2/0 hybridoma cell line in commercial manufacturing processes of recombinant therapeutic proteins is their exogenous lipids requirement for cell proliferation and optimal protein secretion. Lipids are commonly provided to the culture using serum or serum-derivatives, such as lipoprotein supplement. The batch-to-batch variability of these non-chemically defined raw-materials is known to impact cell culture process performance. Lipoprotein supplement variability and its impact on fed-batch production of a recombinant monoclonal antibody (mAb) expressed in Sp2/0 cells were studied using 36 batches from the same vendor. Several batches were associated with early viability drops leading to low process performance during fed-batch production. Increased caspase-3 activity (an indicator of apoptosis) was correlated to viability drops when low-performing batches were used. Addition of an antioxidant to the culture limited the increase in caspase-3 activity. Physicochemical characterization of batches confirmed that lipoproteins are mainly composed of lipids and proteins; no clear correlation between low-performing batches and lipoprotein supplement composition was observed. Controlled lipoprotein oxidation leads to lipoprotein solution browning, increasing absorbance at 276 nm and results in poor process performance. Because low-performing batches absorb more at 276 nm than other batches, oxidized lipids were suspected to be the root cause of low-performing batches. This study increased the understanding of lipoprotein supplement composition, its sensitivity to oxidation and its impact on process performance.

KEYWORDS

antibody titer, apoptosis, lipid oxidation, lipoprotein supplement, raw-material batch variability

1 | INTRODUCTION

Serum and serum-derived supplements such as bovine lipoprotein have been used in cell culture media to stimulate cellular growth in cell and tissue culture by providing critical factors for mammalian cell physiology.[1] Several serum-derived supplements have been used in culture media for human and animal cells in research, biotechnology, and production of recombinant therapeutic proteins, including monoclonal antibodies.[2] Despite potential issues inherent to animal-derived components, lipoprotein supplement are an alternative to serum for supplementation of cholesterol-auxotroph NS-1 and X63 parental cell lines[3]; moreover lipoprotein supplementation was reported to enhance cell growth and antibody secretion of hybridoma[4,5] and more generally mammalian cell lines.[6–9] Lipoproteins are composed of phospholipids, unesterified cholesterol and apolipoprotein surrounding a hydrophobic core of triglycerides and cholesteryl esters.[10] Thus, lipoproteins provide diverse lipids to the culture, as well as antioxidants such as α-tocopherol.[11] However, the amounts of these components in lipoprotein supplements are subject to batch-to-batch variations that may affect the upstream process output.

Serum batch-to-batch variability[2,12–14] and its impact on experimental reproducibility[2,15] with respect to promotion of cell growth and target protein yield[16] are well-known phenomena. However, the impact of lipoprotein supplement variations on hybridoma cell culture has been poorly studied. Lipoproteins being the only source of essential lipids in the cell culture, and lipoprotein supplements being susceptible to contain growth factors, composition variations are susceptible to trigger important process variations. Furthermore, lipoproteins are prone to oxidation. Lipoprotein cholesterol and unsaturated fatty acids oxidation involve a radical mechanism[17] that can be catalyzed by divalent cations such as Cu^{2+}.[18] Lipoprotein oxidation leads to the formation of hydroperoxides,[19] conjugated dienes,[20] oxidized polyunsaturated fatty acids,[21,22] and oxysterols.[23] Overall, lipoprotein oxidation products contribute to cell morphological changes, caspase activity, and DNA fragmentation, all characteristic of apoptosis.[24–28] Oxidized lipoproteins are known for their adverse effects on a wide variety of cell types.[27–32] Since identifying the respective role of each individual compound of oxidized lipoprotein is challenging and often cell line dependent, it is difficult to conclude on the chemical compounds triggering cell changes following contact with oxidized lipoprotein.

Following the observation of a significant variability in cell growth and product titer in correlation with lipoprotein supplement batch change at manufacturing scale of a commercial fed-batch process, an investigation was conducted to identify chemical compounds in the lipoprotein supplement causing variations of process performance. This approach allowed to better characterize the interactions between lipoprotein supplement batch-to-batch variations, apoptosis pathway triggering and process performance. This increased understanding of the relationship between lipoprotein supplement and process performance has allowed the identification of an analytical method able to detect problematic batches before use. All those elements are presented in this article.

2 | MATERIALS AND METHODS

2.1 | Lipoprotein supplement and experimental design

Two lipoprotein supplements manufactured from adult bovine serum were studied, one of which was exclusively manufactured using serum originated from New Zealand (NZ lipoprotein supplement), and the other was manufactured exclusively from serum originated from the United States (US lipoprotein supplement). Twenty-two batches of NZ lipoprotein supplement (batches 1–22) and 14 batches of US lipoprotein supplement (batches 23–36) all from the same vendor were used in this study. Both lipoprotein supplements share a common manufacturing process, only the source of the serum differs. The lipoprotein supplement was stored at 2–8°C. Information obtained from certificates of analysis was gathered.

2.2 | Cell culture and fed-batch experiments

2.2.1 | Cell culture media and feed preparation

A custom proprietary basal medium and other required supplements, including lipoprotein supplement, were dissolved in water for injection (WFI) at 30–35°C with stirring. Media were 0.22 μm filtered aseptically then stored at 2–8°C protected from light. A few hours before their use, cell culture media were incubated at 37°C with 5% CO_2.

Several feed solutions were used according to proprietary process feeding strategy. Glucose, amino acids, trace elements, and lipoprotein supplement were 0.22 μm filtered aseptically and stored protected from light at 2–8°C. The same batch of raw materials was used during media and feed preparations.

Both expansion and production media contain lipoprotein supplement 5.0 mL per liter of cell culture medium.

2.2.2 | Cell line and cell culture

Cell culture experiments were performed with a proprietary cell line, a transfected SP2/0 murine hybridoma cell line producing a recombinant IgG1. The underlying selection principle of the cell line was based on dihydrofolate reductase (DHFR) overexpression allowing the growth of successfully transfected cells in DHFR inhibitor MTX-containing medium. The cell cultures were maintained in an incubator at 37°C in a humidified atmosphere with 10% CO_2 and an orbital agitation of 130 rpm. Shake-flasks were fitted with an airy cap for gas exchange. Studies were performed in triplicate with 40 mL working volume. Cell culture took place in two phases. The first phase is the expansion phase during which the cell line was cultured in the specific batch of cell culture medium to be tested for at least two passages with an interval of 2–3 days between passages. The second phase is the production phase which lasted 11 days. It corresponds to a phase of cell growth and production of the monoclonal antibody. Initial seeding density at production phase was targeted at 2.8×10^5 viable cells/mL. Samples were taken throughout the culture to monitor both cell growth and metabolism.

During the production stage, cell culture was carried out in fed-batch mode with the sequential addition of feed solutions. On the second day of culture, the culture medium was supplemented with lipoprotein supplement at 16.3 mL/L of cell culture medium, this quantity is added to the 5.0 mL/L already present in the medium. The additions of feed solutions were carried out on the second and fourth days of culture after monitoring growth and metabolism.

2.3 | Analytical methods for cell culture

2.3.1 | Process monitoring

In-process control analyses were performed every other day on the cell culture media, starting at working day 0. The viable cell density and the cell viability were measured by Trypan blue exclusion method using a Vi-Cell analyzer (Beckman-Coulter, Brea, CA, USA). Metabolites, such as glutamate, glutamine, lactate, glucose, and ammonium ions, were determined with a Bioprofile Flex2 (Nova Biomedical, Waltham, MA, USA). At the end of cell culture, part of the supernatant was removed, on 0.22 μm filter and stored at −80°C prior to determining the concentration of monoclonal antibody.

2.3.2 | Antibody quantification

Titer quantifications of the IgG1 mAb produced were performed using a Protein A high performance liquid chromatography (PA-HPLC) method. Normalized total productivities were calculated at the end of the cell culture. Normalized volumetric productivity was expressed as a percentage and corresponds to the average of the total volumetric productivity compared to the chosen reference batch.

2.3.3 | Caspase-3 activity

The caspase-3 activity in cell lysate before and after lipoprotein addition was determined using the Caspase-3 Assay kit, product reference: MAK457, (Sigma-Aldrich, St. Louis, MO, USA). Two lipoprotein supplement batches were compared, also the impact of Trolox on Caspase-3 activity was evaluated by adding Trolox 25 mg/L at the beginning of the culture. Trolox is a water-soluble vitamin E analog with antioxidant properties. Cell lysates were sampled right before and 24 h after lipoprotein supplement feed addition. PBS 1× was used as blank sample during the assay.

The assay procedure is adapted from the alternative assay procedure from the Caspase-3 Assay kit Technical Bulletin. One million cells are centrifuged at 500×g for 5 min, 600 μL of RIPA buffer is added to the pellets then the suspension is shaked for 30 min at 4°C. The lysed suspensions are centrifuged at 2500×g for 10 min at 4°C. Fifty microliters of sample lysates and 100 μL of working reagent are added in duplicate to a black flat-bottom 96-well plates. The plate is incubated at 37°C for 60 min in the dark before the fluorescence intensity is measured at $\lambda_{Ex} = 400$ nm/$\lambda_{Em} = 490$ nm.

2.4 | Characterization of Lipoprotein supplement

2.4.1 | Fatty acid

The fatty acid composition of 33 lipoprotein supplement batches was characterized (Millipore Sigma, St. Louis, MO, USA). Fatty acid analyses of lipoprotein supplement were performed on a gas chromatography instrument, SP-2380 capillary GC column, and flame ionization detector (GC-FID). The internal standard approach was used, Supelco 37 Component FAME Mix, product reference: CRM47885 (Supelco, Inc. an affiliate of Sigma-Aldrich, St. Louis, MO, USA).

2.4.2 | Proteomic

Proteomic composition of 31 lipoprotein supplement batches was characterized (Millipore Sigma, Saint Louis, MO, USA). Following trypsin digestion, lipoprotein supplement samples were analyzed by tandem liquid chromatography-mass spectroscopy (LC–MS). Liquid chromatography was performed on an Acquity UPLC Peptide BEH C18 nanoAcquity column and mass spectroscopy on a Fusion instrument (Thermo Fisher, Waltham, MA, USA) equipped with PicoView spray source (New Objective, Littleton, MA, USA). The raw data from LC–MS/MS analysis were submitted and compared to existing data from the Swiss Prot Bovine and Contaminant databases.

2.4.3 | Trace elements

Trace element composition of 15 lipoprotein supplement batches was characterized (Merck Life Science, Darmstadt, Germany). Trace elements analysis was performed using an Inductively Coupled-Plasma Spectrometry (ICP-MS) device. Two methods were used: a semi-quantitative overview analysis Totalquant® (Total Quant Data, Bucuresti, Romania) using quadrupole ICP-MS and a quantitative re-measurement using high-resolution ICP-MS.

2.4.4 | Steroids

Steroid composition of 13 lipoprotein supplement batches was characterized (Millipore Sigma, Saint Louis, MO, USA). An LC–MS/MS workflow for steroid hormones in lipoprotein supplement was adapted from Marta's protocol.[33]

2.4.5 | Sterol

Sterol composition of 12 lipoprotein supplement batches was characterized (Creative Proteomics, Shirley, NY, USA). Sterols present in lipoprotein supplement were extracted twice with methanol and metyl-tert-butyl ether, followed by vacuum evaporation of the extraction solvent. Sterol esters were hydrolyzed in alkaline conditions then extracted with chloroform, and the extraction solvent was removed by vacuum evaporation again. Sterols were resuspended in methanol and analyzed by tandem liquid chromatography-mass spectroscopy (LC–MS). Liquid chromatography was performed on a Shimadzu Prominence HPLC with a Synergi HydroRP-C18 column (Phenomenex, Torrance, CA, USA), and mass spectroscopy on a LTQ-Orbitrap Velos mass spectrometer (Thermo Fisher, Waltham, MA, USA) with a heated electrospray ionization source (HESI).

2.4.6 | Malonaldehyde and 4-hydroxynonenal

Malonaldehyde (MDA) and 4-hyroxynonenal (4-HNE) concentrations in 20 lipoprotein supplement batches were characterized (OXIProteomics, Créteil, France). The quantification of MDA was carried out following manufacturer's instructions using the "Lipid Peroxidation (MDA) Assay Kit (Colorimetric)," product reference: ab233471 (Abcam, Cambridge, UK). Similarly, the quantification of 4-HNE was carried out following manufacturer's instructions using the "Lipid Peroxidation (4-HNE) Assay Kit," product reference ab238538 (Abcam, Cambridge, UK).

2.4.7 | Protein carbonylation

Protein carbonylation of 20 lipoprotein supplement batches was quantified (OXIProteomics, Créteil, France). The quantification of the total proteins was carried out using the Bradford method. Then the extracted proteins were distributed in equal amount per each sample for the following analysis. The oxidized (carbonylated) proteins were labeled with a specific fluorescent probe and then separated by high resolution electrophoresis (SDS-PAGE – gradient 4%–20%).[34] The digital acquisition of images of carbonylated proteins and total proteins was performed using the "iBright" system (Thermofisher). Densitometric analyses of the specific fluorescence signals were performed using "Image J" software (Rasband, WS, Image J, U.S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij, 1997–2014). Carbonylation levels were obtained by normalization of the specific fluorescence signal to the amount of loaded proteins on gel (4 μg) and reported as relative values in comparison to the sample showing the lowest levels of carbonylation (set at 100% as reference). Single independent evaluations were effectuated on different molecular weight sector due to the heterogeneity of signals among the samples.

2.4.8 | UV–visible spectroscopy

Lipoprotein supplement samples were 0.22 μm filtered then added to a 96-well plate that does not absorb in the UV region. Absorbance of lipoprotein supplement samples between 230 and 1000 nm was measured with a SpectraMax i3 spectrophotometer (Molecular Devices, San Jose, CA, USA).

2.5 | Copper-mediated lipoprotein oxidation

The relation between lipid oxidation products present in oxidized lipoprotein and the cytotoxic effect of oxidized lipoproteins is a well-known phenomenon.[35] Because copper acts as a catalyst of the lipid peroxidation reaction, it is possible to use copper sulfate to produce oxidized lipoproteins and study their potential cytotoxic effect on cultured cells.[19]

A 0.22 μm aseptically filtered 0.003 M stock solution of $CuSO_4$, "Copper (II) Sulfate, anhydrous, powder 9", product reference: 461657 (Sigma-Aldrich, St. Louis, MO, USA) in reverse osmosis water was prepared and added to lipoprotein supplement samples for oxidation. Lipoprotein supplement samples containing various concentrations of copper (from 0 to 375 μM) were oxidized over different periods of time (from 0 to 72 h) and incubated at different temperatures (4°C, 20°C, and 37°C) to study the impact of all three parameters on oxidation-mediated toxicity. Cell culture media and feed solutions were then prepared using previously mentioned oxidized lipoprotein supplement samples. Control conditions were run in parallel containing unoxidized lipoprotein supplement but supplemented with $CuSO_4$ during the inoculation.

2.6 | Data analysis

Data were first assembled in Excel 2018 (Microsoft, Redmond, WA). JMP® 16 statistical software (SAS Institute Inc., Cary, NC, USA) and SIMCA® 17 multivariate analysis software (Umetrics by Sartorius, Goettingen, Germany) were used for multivariate data analysis.

2.6.1 | Integral viable cell

The integral viable cell (IVC) quantifies the effective working time for a dynamic viable cell concentration within a given frame of time. It was approximated using the trapezoid approximation between every VCD measurements.

2.6.2 | Population doubling level

The population doubling level (PDL) allows the comparison of cumulative cell expansion passages without bias due to distinct initial and final cell concentrations. The PDL was calculated as follows:

PDL = PDL0 + 3,322*($\log C_f - \log C_i$) with C_i and C_f being the viable cell concentration at the beginning and the end of each cell expansion passage.

3 | RESULTS

3.1 | Impacts of lipoprotein supplement batch-to-batch variability on cell growth and mAb production

Impact of lipoprotein supplement batch-to-batch variability on cell growth was investigated using 36 batches of lipoprotein supplement. The batch-to-batch variability of lipoprotein supplement was found to negatively impact the evolution of viable cell density (VCD), Figure 1a, and cell viability after feed addition, Figure 1b. Several batches had detrimental effects on VCD (batches 21 and 22 in Figure 1), indeed the VCD peak of these batches was near half the VCD peak of other batches. Moreover, these batches are associated with earlier viability drops than other batches. Interestingly, the differences between the batches that induce early viability drops and other batches appeared after WD02, after feed addition of the lipoprotein supplement. The direct impact on cell culture performance of lipoprotein supplement batches that induced viability drops translated in a reduced metabolism visible on glucose, Figure 1c, glutamine, Figure 1e, and ammonium consumption, Figure 1g, but without significant impact on lactate, Figure 1d, and glutamate production, Figure 1f. Glutamine concentration was below the limit of detection after days 9 and 11 for batches 7 and 30, and only after day 11 for batch 29. These differences in glucose and glutamine consumption could be related to the effect of lipoprotein supplement on viable cell density.

The average volumetric productivity observed for 36 batches of lipoprotein supplement relative to the chosen reference batch is presented in Figure 2. The productivity varied from −85% to +11% compared to the reference batch. Only NZ lipoprotein supplement, batches 11–22, was associated with drastic productivity drops up to −85%, whereas batch-to-batch variability of US lipoprotein supplement, batches 23–36, triggered productivity variations from −21% and +4%. Due to the low productivity associated with batches 11–22, these batches were assigned as low-performing batches.

The IVC density at the end of the culture was calculated for each batch, each IVC was normalized by a comparison with the reference batch. The relationship between the normalized mAb titer and the normalized IVC at the end of the culture for each batch is presented in Figure 3. Low-performing batches, from −60% to −85% relative mAb titer, are characterized by a low IVC synonym to low cell growth. The good correlation between IVC and titer indicates that the specific productivity of the cells is not affected by low-performing batches.

Because expansion medium contains lipoprotein supplement 5.0 mL/L, the relation between lipoprotein supplement concentration and cell growth during cell expansion was investigated. Two lipoprotein supplement batches were tested: the reference batch 7 (relative productivity 0%) and a low-performing one, batch 11 (relative productivity −80%). Six lipoprotein supplement concentrations were tested: 5.0, 8.2, 11.4, 14.6, 17.8 and 21.0 g/L. The impact of lipoprotein supplement concentration on cell growth was investigated through four 2–3 days cell expansions, PDL are presented in Figure 4.

The impact of lipoprotein supplement concentration on cell growth depended on the lipoprotein supplement batch used. When the reference was used, lipoprotein supplement concentration between 5.0 and 21.0 g/L had no impact on PDL. By contrast, when the low-performing batch was used, lipoprotein supplement concentration between 5.0 and 11.4 g/L had no impact on PDL whereas between 14.6 and 21.0 g/L the lipoprotein supplement concentration was negatively correlated with PDL.

The low-performing batch is associated with lower PDL than the reference batch between 11.4 and 17.8 g/L, the difference in term of cell growth is even more significant at 21.0 g/L meaning that there is a dose–response relationship between lipoprotein supplement concentration and reduced cell growth for the low-performing lipoprotein supplement batch.

3.2 | Impact of lipoprotein supplement oxidation on cell culture process

In order to investigate whether lipoprotein supplement oxidation was causing low-performing batches, the impact of lipoprotein oxidation on batch performance was assessed. Controlled lipoprotein supplement oxidation was conducted using $CuSO_4$ as a catalyst. Lipoprotein supplement browning was observed during $CuSO_4$-mediated oxidation in Figure 5. Moreover, oxidized batches negatively impacted cell growth and viability with regards to their degree of oxidation, Figure 6. Depending on the conditions of oxidation, and therefore the degree of oxidation, oxidized lipoprotein supplement either decreased cell growth but not viability, decreased cell growth and viability or nearly killed the entire culture. $CuSO_4$ itself was not causing decreased cell growth and viability drops, as controls with $CuSO_4$ addition in the culture (without pre incubation in the cell culture medium) did not show decreased performance (Figure 6): Reference batch—8 h at 37°C 0 μM $CuSO_4$ then addition of 150 μM $CuSO_4$ during inoculation.

Lipoprotein supplement is prone to oxidation, and oxidized lipoprotein supplement negatively impacted the process performance. Such lipoprotein oxidation is catalyzed by divalent cations.[18,19] Moreover, storage at 37°C of the lipoprotein supplement for more than 24 h led to a gradually decreasing process performance (Figure 7). This indicates that the lipoprotein supplement is deteriorating during incubation even in absence of divalent ions that act as catalysts.

3.3 | Caspase-3 activity measurement

Since low-performing lipoprotein supplement batches were characterized by an early viability drop following lipoprotein feed addition during the production phase, caspase-3 activity was measured to

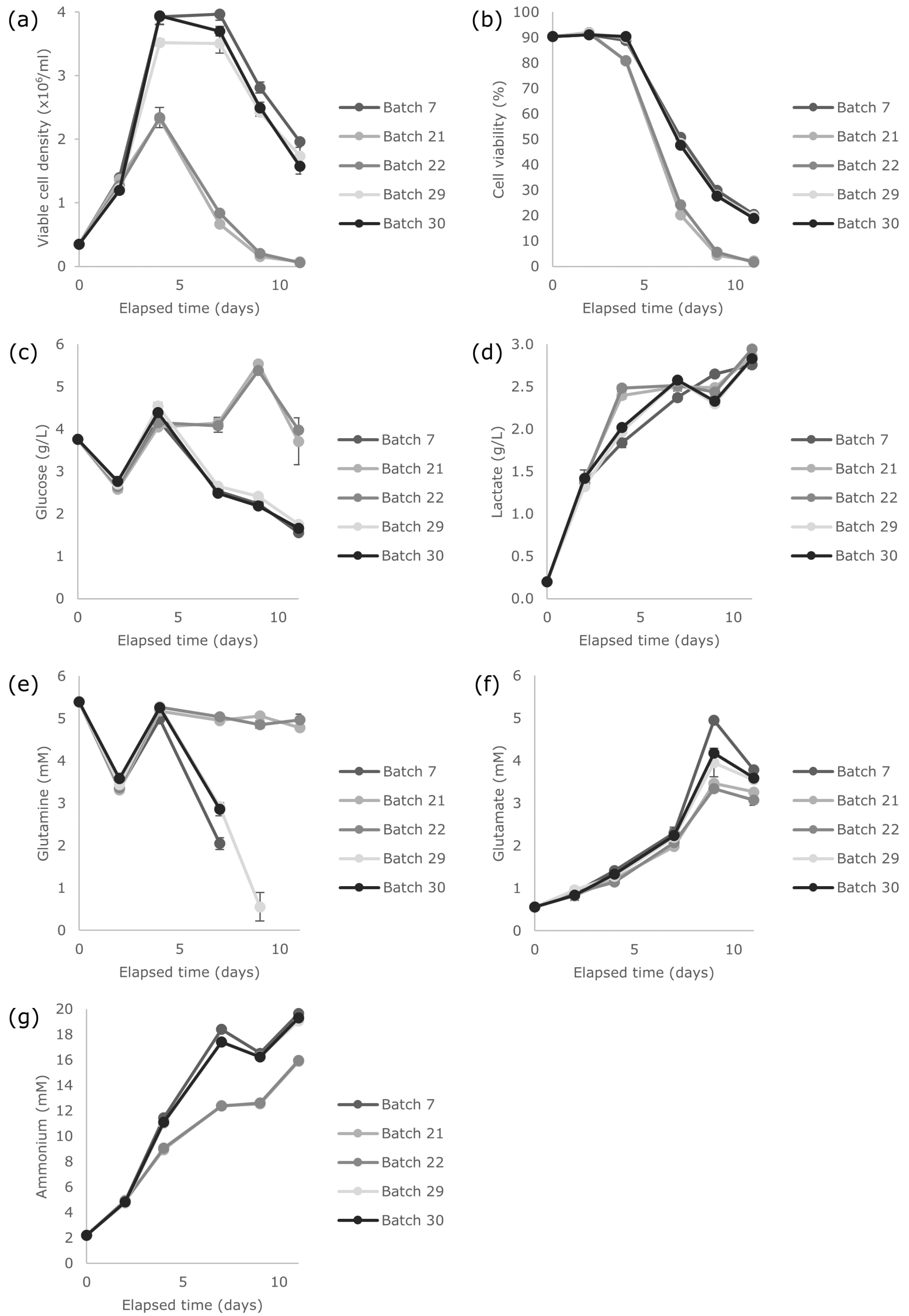


FIGURE 1 Impact of lipoprotein supplement batches in cell culture media and feed solution on cell density (a), cell viability (b), glucose (c), lactate (d), glutamine (e), glutamate (f), and ammonium (j). Each batch of Lipoprotein supplement was tested in triplicate in shake-flasks. On the second day of culture, the culture medium was supplemented with a feed solution containing glucose and glutamine and a second feed that contains the same batch of pure lipoprotein supplement that was used for media preparation. Mean ± standard deviation ($n = 3$).

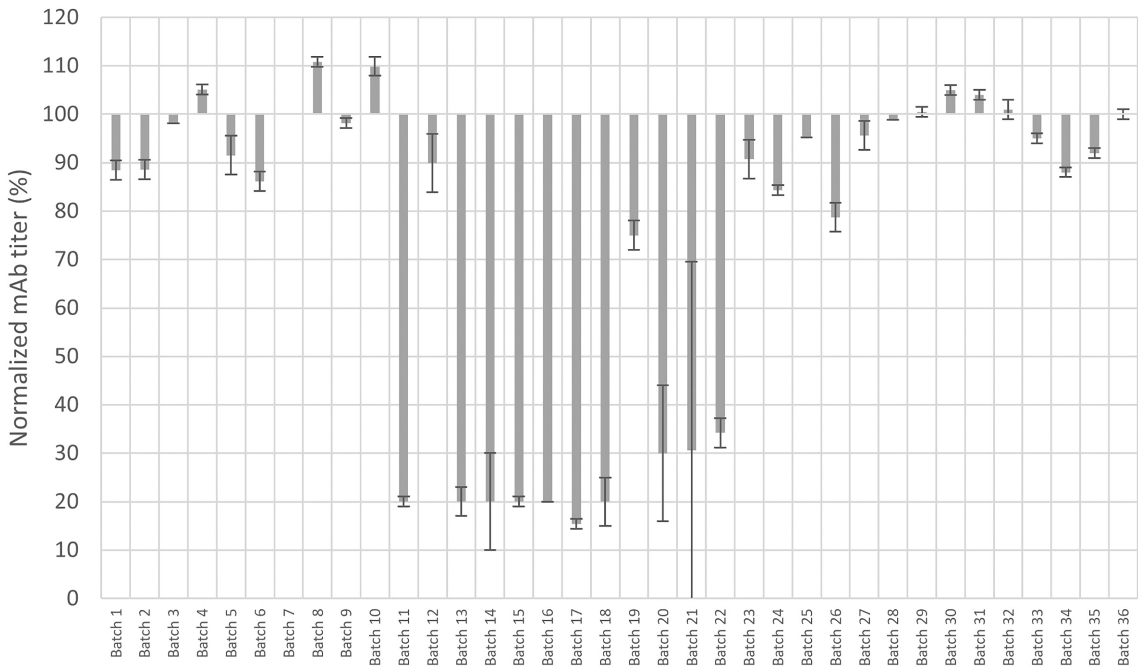
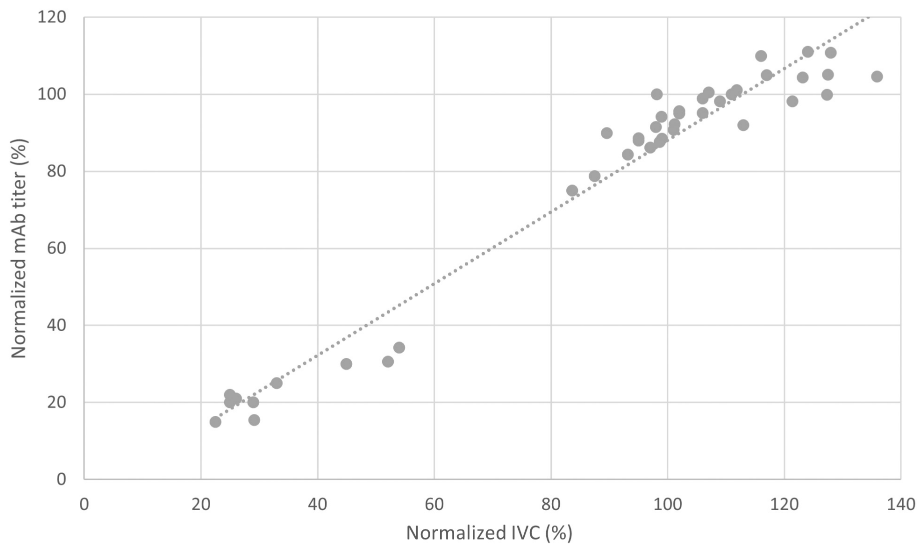


FIGURE 2 Impact of batch-to-batch variability of lipoprotein supplement on monoclonal antibody titer. Thirty-six batches of lipoprotein supplement from the same vendor were compared to a given batch for which the mAb titer was fixed at 100%. Tests were performed in shake-flasks in triplicate. Results are expressed as the percentage of the titer obtained with a given lipoprotein supplement batch at the end of the culture compared to the titer obtained with the reference batch. Mean ± standard deviation from three independent cultures.

FIGURE 3 Fit of normalized mAb titer by normalized IVC at the end of the culture. The correlation between normalized mAb titer and normalized IVC is statistically significant ($p < 0.0001$) with $R^2 = 0.96$.



determine whether apoptosis was causing the observed viability drops. Again, two lipoprotein supplement batches were tested: the reference batch 7 (relative productivity 0%) and a low-performing one, batch 11 (relative productivity−80%). Each batch was tested in absence and in presence of Trolox 25 mg/L. Trolox is a water-soluble analog of vitamin E known for its antioxidant properties,[36] the prevention of lipid peroxidation[37] and the prevention of oxidative stress-induced apoptosis.[38] Since oxidative stress and oxidized lipids are known to trigger cell death through apoptosis and ferroptosis pathways,[39] addition of an antioxidant to the culture was tested to evaluate the role of oxidative stress on caspase-3 activity. All cell culture experiment took place in triplicate and all fluorescence measurement in duplicate. The average sample fluorescence intensity of every duplicate was calculated, then the sample fluorescence intensity value from the blank, PBS 1×, was subtracted to every values. The relative caspase-3 activity before and after feed addition was estimated by comparison of the fluorescence intensities.

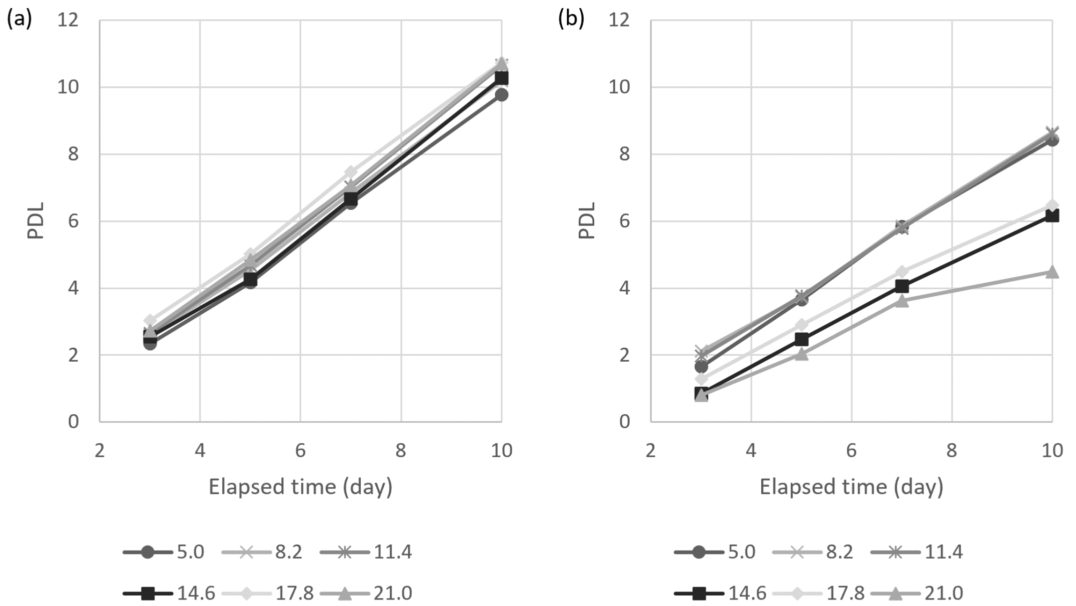


FIGURE 4 PDL during four 2–3 days cell expansions at different lipoprotein supplement concentrations between 5.0 and 21.0 g/L using lipoprotein supplement reference batch 7 (a) and low-performing batch 11 (b). By contrast with the reference batch, when the low-performing batch concentration increased above 11.4 g/L the PDL starts to decrease with lipoprotein supplement concentration.

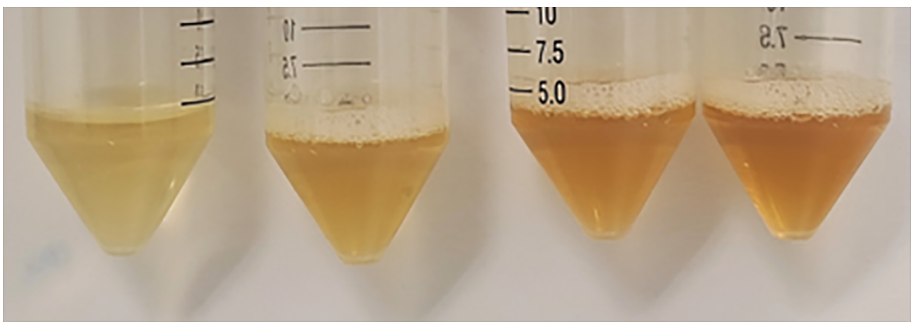


FIGURE 5 Lipoprotein supplement browning after 24 h of $CuSO_4$-mediated oxidation at 37°C, 320 rpm. The samples were oxidized with various $CuSO_4$ concentrations, from left to right 0, 50, 100, and 150 μM.

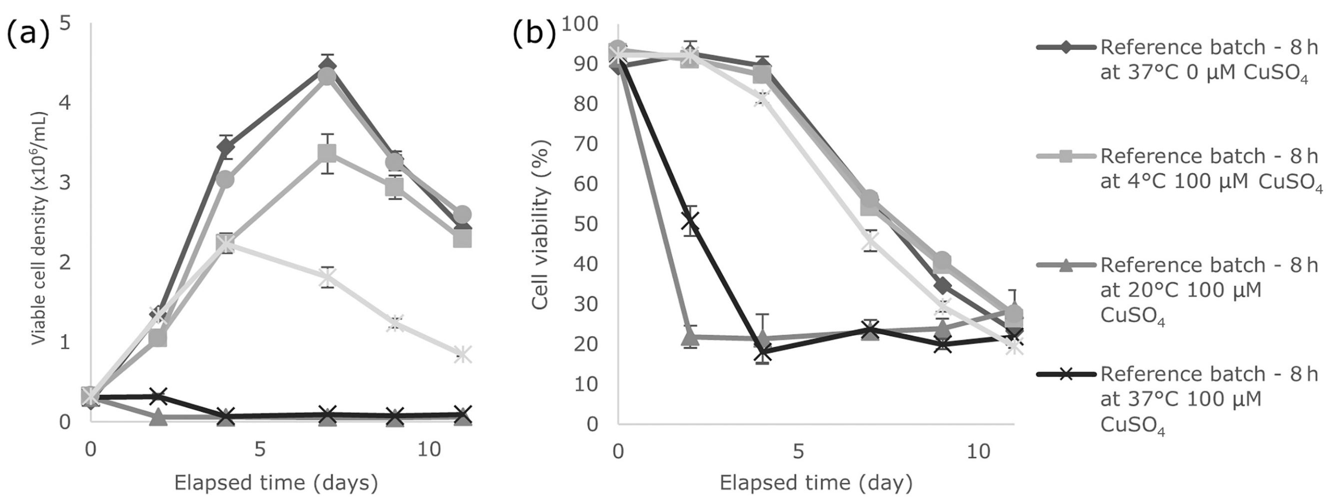


FIGURE 6 Effect of lipoprotein supplement oxidation on cell growth (a) and viability (b) during the production phase. Mean ± standard deviation from three independent cultures.

The low-performing lipoprotein supplement batch was characterized by an increase of caspase-3 activity 24 h after feed addition by approximately +220%, whereas feed addition of the reference batch increased by +37%, Figure 8. Hence, we concluded that some compounds present in the low-performing batch and not in the reference batch might trigger apoptosis pathway leading to caspase-3 activation. Coherently, these compounds triggering apoptosis may explain observed viability drops.

In parallel with the comparison between the reference and low-performing batches, similar tests were conducted with cultures supplemented with Trolox 25 mg/L, Figure 8. In absence of Trolox the impact of reference batch feed addition on caspase-3 activity was similar to the presence of Trolox, with caspase-3 activity increase by +37% and +42% respectively. However, in presence of Trolox 25 mg/L the impact of low-performing batch feed addition on caspase-3 activity was only a +73% increase by comparison with the +220% increase in absence of Trolox. In conclusion, oxidative compounds are potential candidate to explain low-performing batches-related viability drops.

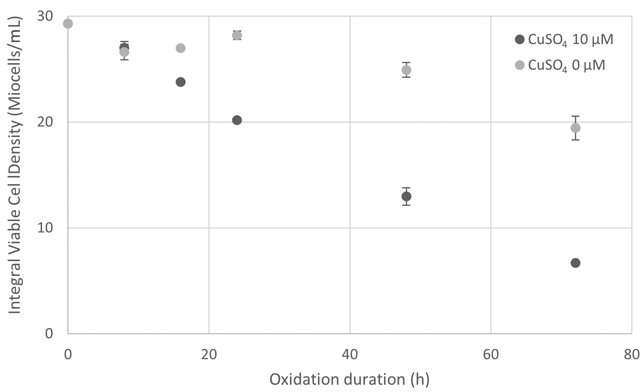


FIGURE 7 Effect of $CuSO_4$ concentration during lipoprotein supplement oxidation. Addition of $CuSO_4$ during lipoprotein supplement oxidation increased the kinetic of oxidation, the degree of oxidation and so the IVC at the end of the production phase. Note that lipoprotein supplement degradation occurred in absence of $CuSO_4$ leading to the gradual decrease in IVC. Mean ± standard deviation from three independent cultures.

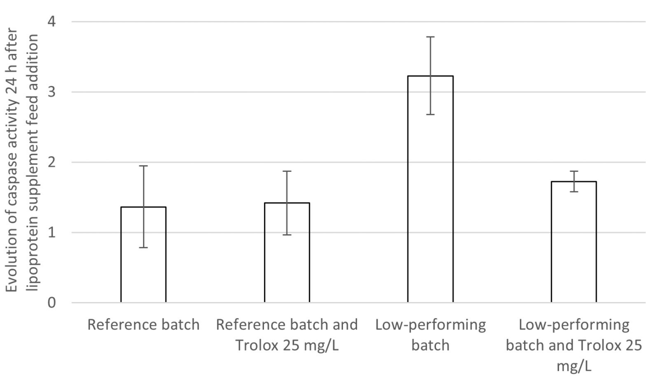


FIGURE 8 Effect of lipoprotein supplement feed addition and addition of Trolox 25 mg/L in the medium on caspase-3 activity. Relative caspase-3 activity was estimated by a comparison of fluorescence intensity measured with Caspase-3 assay kit. Mean ± standard deviation from two independent cultures and two caspase-3 activity measurements of each culture.

3.4 | Characterization of lipoprotein oxidation by-products

3.4.1 | Influence of the sterol composition of lipoprotein supplement on process performance

Because oxidized lipoprotein cytotoxicity properties are commonly attributed to oxysterols, cholesterol oxidation by-products, the sterol composition of 12 lipoprotein supplement batches was characterized. Total sterol content results are presented in Table 1 and variations of sterol composition are presented in Figure 9.

Lipoprotein supplement contains not only cholesterol but also cholesterol oxidation derivatives. Cholesterol oxidation derivatives can be divided into two categories: first, oxysterols: molecules derivates from cholesterol by addition of one or two oxygen molecules and second: oxidized forms of cholesterol without oxygen addition: cholestenone, desmosterol, and cholestanol.

No difference of sterol composition was observed between United States and NZ lipoprotein supplements (data not shown).

The principal component of the sterol composition of lipoprotein supplement, presented in Figure 10, shows that low-performing batches form a cluster distinct from other batches. According to the PCA presented in Figure 10 and the charts presented in Figure 11:

- Cholesterol content is similar between the low-performing batches and the other batches, Figure 11a;
- Low-performing batches contain less oxidative derivatives of cholesterol, especially cholestenone, Figure 11b;
- Low-performing batches contain more desmosterol, Figure 11c.

Low-performing batches do not contain more cholesterol oxidation derivatives than other batches, surprisingly, they contain less cholestenone than other batches.

Lastly, low-performing batches contain more desmosterol than other batches. If desmosterol has a negative impact on SP2/0 cell growth it could partially explain the low growth observed with low-performing batches. However, ×63 mouse myeloma cells, the parental cell line of SP2/0, are able to metabolize cholesterol precursors such as desmosterol; indeed desmosterol supports the growth of ×63 cells.[40] Consequently, higher level of desmosterol in low-performing batches is not sufficient to explain growth issues related to these batches.

3.4.2 | Influence of malonaldehyde and 9-hydroxynonenal content in lipoprotein supplement on process performance

N-3 and n-6 polyunsaturated fatty acids peroxidation by hydroxyl radicals leads to the formation of α,β-unsaturated lipid aldehydes

TABLE 1 Variations of sterol content of lipoprotein supplement.

	All lipoprotein batches	US lipoprotein batches	NZ lipoprotein batches	Low-performing NZ lipoprotein batches	Standard NZ lipoprotein batches
Sterol content (g/L lipoprotein)	12.3 ± 4.9	13.0 ± 5.0	12.0 ± 4.8	11.5 ± 5.0	12.7 ± 4.2

Note: Mean ± standard deviation ($n = 12$).

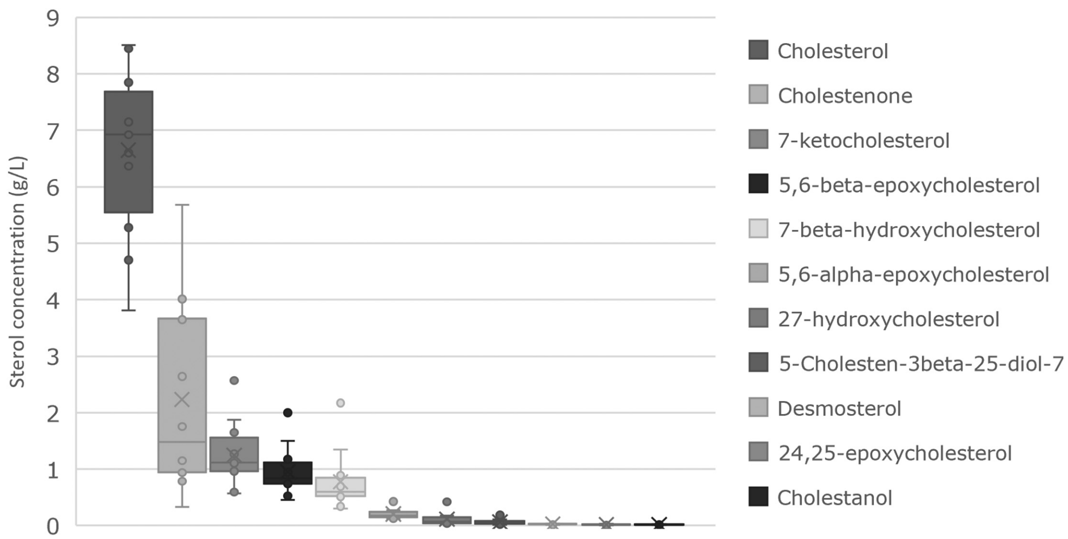


FIGURE 9 Variations of sterol composition of 12 lipoprotein supplement batches. Mean ± standard deviation ($n = 12$).

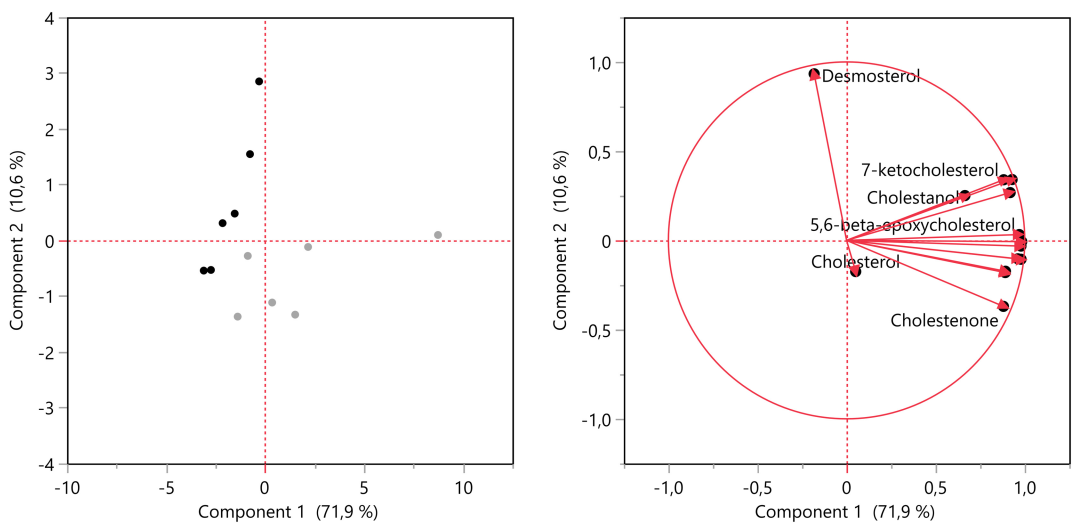


FIGURE 10 Relation between component 1 and component 2 of the principal component analysis of the sterol composition of 12 lipoprotein supplement batches. Low-performing batches are in black whereas other batches are in gray.

such as 4-hydroxynonenal (4-HNE), 4-hydroxyhexenal, malondialdehyde (MDA), and acrolein.[39] Two lipid peroxidation by-products MDA and 4-HNE were characterized in 20 lipoprotein supplement batches. Results are presented in Table 2. Only slight variations of MDA content were observed among batches. US lipoprotein supplement contains considerably more 4-HNE than NZ lipoprotein supplements. Lastly, important variations of 4-HNE content were observed among United States and standard NZ lipoprotein supplement batches.

Low-performing lipoprotein supplement batches were found to contain less 4-HNE and slightly more MDA (+11%) than other batches. The presence of MDA and 4-HNE in every lipoprotein supplement batches demonstrates that lipid peroxidation of lipoprotein supplement components has occurred. Even if low-performing batches contain more

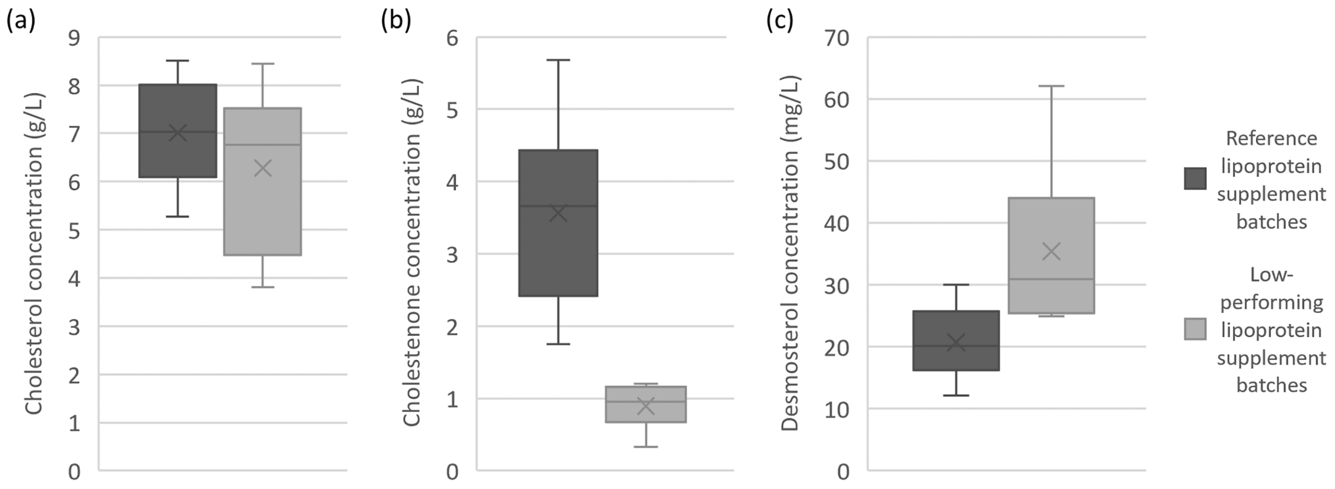


FIGURE 11 Comparison of the cholesterol (a), cholestenone (b) and desmosterol (c) concentrations in low-performing and standard lipoprotein supplement batches. Mean ± standard deviation ($n = 12$).

TABLE 2 Variations of MDA and 4-HNE content in lipoprotein supplement.

	All lipoprotein batches	US lipoprotein batches	NZ lipoprotein batches	Low-performing NZ lipoprotein batches	Standard NZ lipoprotein batches
MDA (μg/mL)	1.36 ± 0.34	1.22 ± 0.20	1.44 ± 0.39	1.51 ± 0.47	1.35 ± 0.29
4-HNE (μg/mL)	47.62 ± 44.56	82.96 ± 43.93	22.77 ± 19.16	12.85 ± 5.58	36.67 ± 23.43

Note: Mean ± standard deviation ($n = 20$).

MDA than other batches on average, it cannot explain the detrimental effects of these batches because few batches that perform well contain high levels of MDA. Because MDA and 4-HNE negatively impact cell growth, viability, and even trigger apoptosis,[41,42] batch-to-batch variations of MDA and 4-HNE content can partially explain the process performance variations observed but not the important negative impact on viability of low-performing batches.

3.4.3 | Influence of protein carbonylation in lipoprotein supplement on process performance

MDA, 4-HNE, and other reactive aldehydes formed during lipid peroxidation react with protein, leading to protein carbonylation and protein structure alteration.[39] Such modifications induce loss of protein–protein interactions and decrease of protein catalytic activity, both source of detrimental effects for the cell. Protein carbonylation level was measured in 20 lipoprotein supplement batches. Protein carbonylation was measured in three fractions: protein with a molecular weight less than 20 kDA, between 20 and 50 kDA, and between 50 and 100 kDA. Results are presented in Table 3.

Only slight variations of protein carbonylation levels were observed between batches. No higher protein carbonylation levels were observed in low-performing lipoprotein supplement batches. Multivariate analysis of protein carbonylation results showed no correlation between the protein carbonylation levels and lipoprotein supplement source or batch performance. Protein carbonylation, MDA, and 4-HNE characterization results are concordant: lipid peroxidation

TABLE 3 Variations of protein carbonylation in lipoprotein supplement.

	All lipoprotein batches	US lipoprotein batches	NZ lipoprotein batches	Low-performing NZ lipoprotein batches	Standard NZ lipoprotein batches
Carbonylation level of protein <20 kDA (RFU/μg)	4725 ± 1341	4775 ± 1359	4685 ± 1390	4381 ± 1089	5217 ± 1866
Carbonylation level of protein 20–50 kDA (RFU/μg)	3303 ± 810	3660 ± 1040	3012 ± 415	3181 ± 295	2715 ± 464
Carbonylation level of protein 50–100 kDA (RFU/μg)	2979 ± 787	3103 ± 814	2878 ± 789	2736 ± 600	3126 ± 1107

Note: Carbonylation levels unit is the specific fluorescence signal per microgram of loaded proteins on gel. Mean ± standard deviation ($n = 20$).

occurs in lipoprotein supplement and lipid peroxidation by-products react with proteins leading to protein carbonylation. Despite slight variations of protein carbonylation levels among batches that can partially explain the process performance variations observed, the protein carbonylation of low-performing NZ lipoprotein supplement batches is not different enough from other batches to explain the important negative impact on viability of these batches.

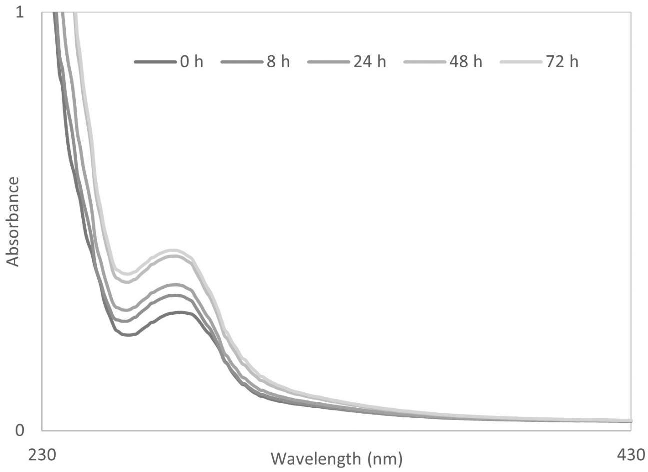


FIGURE 12 UV–visible absorbance spectra of 100× diluted oxidized lipoprotein supplement batch 7 after different oxidation durations at 37°C in absence of $CuSO_4$. Step between each measurement: 1 nm.

3.5 | Analytic tools to detect lipoprotein supplement oxidation

3.5.1 | Absorbance: a marker of lipoprotein supplement oxidation

Since lipoprotein supplement oxidation was associated with solution browning, UV–visible analysis of lipoprotein supplement batches, including $CuSO_4$-oxidized samples, were conducted. Lipoprotein supplement oxidation was correlated with an increase of solution absorbance at 276 nm (Figure 12). Absorbance at 276 nm was well correlated with oxidation duration and so the degree of lipoprotein supplement oxidation (Figure 13).

Lipoprotein supplement incubation at 37°C in presence of oxidative atmosphere for a period from 24 to 72 h was correlated with an increase of solution absorbance at 276 nm. Incubation in similar conditions but in presence of $CuSO_4$ was also correlated with an increase of solution absorbance at 276 nm. A response surface model taking into account incubation duration and $CuSO_4$ concentration was able to explain 74% of the absorbance at 276 nm variations observed during lipoprotein supplement oxidation, Figure 13. Thus, absorbance at

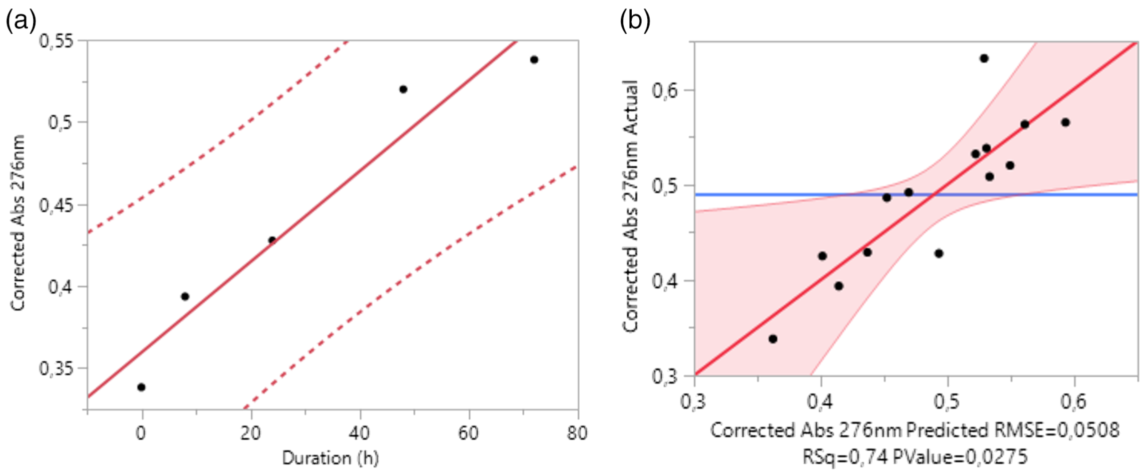


FIGURE 13 (a) Fit of the corrected absorbance at 276 nm of oxidized lipoprotein supplement by the oxidation duration at 37°C in absence of $CuSO_4$. Corrected absorbance was calculated by subtraction of the blank absorbance to the sample at 276 nm. The correlation between the oxidation duration and the corrected absorbance at 276 nm is statistically significant ($p = 0.0068$) with $R^2 = 0.94$. Confidence curve $_\alpha = 0.05$ (b) Comparison of the corrected absorbance at 276 nm with the predicted by an OPLS of oxidation duration (h) and $CuSO_4$ (μM). $R^2 = 0.74$. Confidence curve $\alpha = 0.05$.

TABLE 4 Variations of absorbance at 276 nm ok lipoprotein supplement.

	All lipoprotein batches	US lipoprotein batches	NZ lipoprotein batches	Low-performing NZ lipoprotein batches	Standard NZ lipoprotein batches
Absorbance at 276 nm	0.510 ± 0.094	0.443 ± 0.058	0.580 ± 0.070	0.601 ± 0.069	0.519 ± 0.018

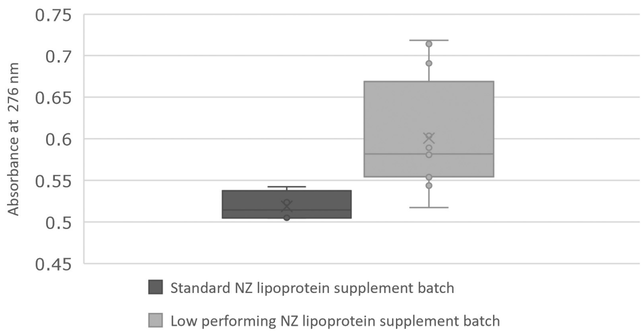


FIGURE 14 Comparison of the absorbance at 276 nm of low performing NZ batches with other NZ batches.

276 nm is an accurate parameter to monitor lipoprotein supplement oxidation.

3.5.2 | Fingerprinting of lipoprotein supplement batches

The absorbance at 276 nm of 33 lipoprotein supplement batches was measured to evaluate absorbance measurement as a fingerprinting tool, Table 4.

Overall, NZ lipoprotein supplement absorbs more than US lipoprotein supplement, moreover low-performing lipoprotein supplement batches absorb more than other NZ lipoprotein supplement batches, Figure 14.

Distribution of absorbance at 276 nm measurements of low-performing and standard-performing NZ batches indicates that the upper 95% mean of the standard-performing NZ batches is 0.547 and the lower 95% mean of the low-performing NZ batches is 0.557. Consequently, it is possible to state with 95% confidence that lipoprotein supplement batches with an absorbance at 276 nm higher than 0.557 are low-performing batches. Among the 33 lipoprotein supplement batches tested, only one US and one NZ batches are conflicting with this assumption.

4 | DISCUSSION

Lipoprotein supplement is added to hybridoma cell culture media in order to provide lipids. Such lipid supplementation is essential to enhance cell growth and monoclonal antibody secretion in many fed-batch processes. In our hands, several lipoprotein supplement batches were associated with low-process-performance, from −45% to −85% titer at the end of the culture compared to a reference batch. The root-cause was investigated to obtain a better understanding on the impact of raw-material variations on process performance and to determine, why these low-performing batches were so detrimental to the process.

Our analysis revealed that below 10 g/L lipoprotein supplement in the cell culture, low-performing batches behaved inconspicuously. However, above 10 g/L, low-performing batches were associated with lower PDL during the expansion phase and with earlier viability drops during the production phase both indicating a negative impact on cell growth. Because the negative impact on cell growth appears only at high lipoprotein supplement concentrations, the lack of essential compounds for cell growth and maintenance in these batches is unlikely to explain the negative impact on cell growth. However, the dose–response relation between low-performing lipoprotein supplement batches and the negative impact on cell growth was indicative of the presence of toxic compounds hampering cell growth.

The presence of toxic compounds in low-performing batches was indirectly confirmed by the massive caspase-3 activity increase (+223%) following lipoprotein supplement feed addition compared to the reference batch (+37%). Since caspase-3 activity is an indicator of apoptosis, it is not surprising that batches that are associated with earlier viability drops also displayed enhanced caspase-3 activity. Interestingly, addition of Trolox 25 mg/L had no significative impact on the increase of caspase-3 activity following feed addition of the lipoprotein supplement reference batch (+42%) but it reduced the increase of caspase-3 activity associated with a low-performing batch from +223% to 73%. These results suggest that some compounds present in low-performing batches trigger an apoptosis pathway, and their action is partly inhibited by Trolox. Trolox is known for its antioxidant properties,[36] the prevention of lipid peroxidation[37] and the prevention of oxidative stress-induced apoptosis.[38]

Oxidized lipoproteins are well known for their ability to trigger apoptosis,[27–32] thus lipoprotein supplement oxidation was studied to evaluate its sensitivity to oxidation and its impact on process performance. Lipoprotein supplement oxidation, via storage at 37°C in absence of $CuSO_4$, or in presence of $CuSO_4$[19] resulted in lipoprotein supplement browning, indicating a chemical reaction, and negatively impacted process performance. Media supplementation with such oxidized lipoprotein batches resulted in decreased cell growth and triggered viability drops, leading to low process productivity. Moreover, oxidation duration, oxidation temperature and $CuSO_4$ concentration were correlated closely to both solution browning and a negative impact on process performance. Because the lipoprotein oxidation method using $CuSO_4$ leads to the formation of hydroperoxide- or oxysterol-rich oxidized lipoproteins,[19] both hydroperoxide by-products and oxysterols were quantified in lipoprotein supplement batches to determine whether these compounds are causing the low performance of some batches. Even if main hydroperoxide by-products, 4-hydroxynonenal, and malonaldehyde, as well as several oxysterols were detected in all lipoprotein supplement batches, they were not present in higher quantity in low-performing batches. Furthermore, protein carbonylation, the reaction of hydroperoxide with protein side chains amino acids was measured. Again, protein carbonylation was detected in all lipoprotein supplement batches but not in higher quantity in low-performing batches. However, the analysis of protein carbonylation level was a quantitative measurement and it did not consider which protein were carbonylated. Indeed, the nature of proteins that have been carbonylated is susceptible to considerably affect how lipoprotein oxidation will have an impact on the culture.

Taken together, all these analytical results indicate that all lipoprotein supplements irrespective on their influence on process performance contain oxidized compounds to some extent, but no correlation between the concentration of oxidized forms and low process performance was detected.

Lastly, because lipoprotein supplement browning was observed during lipoprotein supplement oxidation, UV–visible measurements of lipoprotein supplement batches and $CuSO_4$-mediated oxidized lipoprotein supplements were made. Absorbance at 276 nm was positively correlated with oxidation duration and so the degree of lipoprotein supplement oxidation. These results suggest that oxidation products absorbing at 276 nm are formed during lipoprotein oxidation. Based on the distribution of absorbance at 276 nm of 33 lipoprotein supplement batches, an absorbance limit that distinguishes low-performing batches from other batches was identified. It was stated with 95% confidence that after dilution 100× with water, lipoprotein supplement batches with an absorbance at 276 nm higher than a certain threshold value, in this case 0.557 are low-performing batches. This detection method would have been able to detect 9 of the 10 low-performing lipoprotein supplement batches and only one batch would have been flagged wrongly as low-performing batch.

In conclusion, oxidized lipoprotein supplements negatively impact cell growth, cell viability and process performance similarly to low-performing lipoprotein supplement batches. Moreover, absorbance measurement at 276 nm was an accurate method to follow lipoprotein supplement oxidation and to distinguish low-performing batches from other batches.

Surprisingly, oxidized lipoprotein supplements and low-performing batches share common properties, but major lipoprotein oxidation by-products were not present in higher quantity in low-performing batches than in other batches. Studies of oxidized lipoprotein cytotoxicity often concluded that oxysterols,[29,43–45] especially 7-ketocholesterol,[46,47] or lipid hydroperoxide[48,49] are mostly responsible for oxidized lipoprotein toxicity. Spiking of oxysterols in lipoprotein supplement has confirmed that these compounds are toxic for hybridoma cells used in our process (data not shown). If lipoprotein solution oxidation is the source of toxic compounds present in low-performing batches, they are not the compounds commonly identified as causing oxidized lipoprotein toxicity, such as oxysterol, lipid hydroperoxides and associated by-products. Hence, it is tempting to speculate that other oxidation products that escaped our attention so far are the main cause of the observed differences in process performance. Alternatively, because lipoprotein uptake by mammalian cells is a well-regulated mechanism involving cell surface receptor and lipoprotein recognition,[50] lipoprotein supplement oxidation could have altered apolipoprotein involved in lipid recognition leading to lipoprotein uptake dysregulation. Likewise, oxidation of other components present in the lipoprotein supplement matrix in addition to lipoproteins, such as bovine serum albumin could have an impact on process performance. Lastly, growth factors, antioxidants and bioactive complex lipids could be present in lipoprotein supplement, and these potential growth promoting compounds may eventually become growth inhibitors after oxidation. Further investigation could start by the spiking of caspase-3 inhibitors to investigate whether activation of the apoptosis pathway is the main cause or the result of low batch performance.

Despite not being able to identify the precise chemical compounds, raw-material degradation, or alteration causing the low-performing lipoprotein supplement batches, this investigation has increased the understanding on lipoprotein supplement composition, its sensitivity to oxidation and its impact on process performance. A fingerprinting method, based on UV spectroscopy, able to monitor lipoprotein oxidation and to detect with confidence low-performing lipoprotein batches has been proposed. Because UV measurement is an easy to implement method, which is also more cost and time effective than cell culture experiments, it could be applied to avoid oxidized batches and ensure the robustness of cell culture process involving lipoprotein supplements.

AUTHOR CONTRIBUTIONS

Rémy Moisant: Data curation (lead); formal analysis (lead); investigation (lead); methodology (equal); writing – original draft (lead); writing – review and editing (equal). **Elliott Cowles:** Data curation (equal); formal analysis (equal); investigation (equal). **Léonie Broutel:** Data curation (supporting); investigation (supporting). **Véronique Deparis:** Investigation (equal); methodology (equal); supervision (equal); writing – review and editing (equal). **Anthony Baud:** Investigation (equal); supervision (equal); writing – review and editing (equal). **Joerg von Hagen:** Supervision (equal); writing – review and editing (equal). **Harald Kolmar:** Supervision (equal); writing – review and editing (equal).

ACKNOWLEDGMENT

The authors thank analytical services from Millipore Sigma in Saint Louis, USA, from Merck KGaA in Darmstadt, Germany, from OxiProteomics and from Creative Proteomics for the completion of the physico-chemical analyses on lipoprotein supplements.

FUNDING INFORMATION

This study was entirely funded by Merck Serono SA (an affiliate of Merck KGaA, Darmstadt, Germany).

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/btpr.3372.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Rémy Moisant https://orcid.org/0000-0002-5695-639X

REFERENCES

1. Jenkins N, Castro P, Menon S, Ison A, Bull A. Effect of lipid supplements on the production and glycosylation of recombinant interferon-γ expressed in CHO cells. *Cell Culture Eng*. 1994;15(1–3): 209-215.
2. van der Valk J, Bieback K, Buta C, et al. Fetal bovine serum (FBS): Past – present – future. *ALTEX*. 2018;35(1):99-118.
3. Sato JD, Kawamoto T, McClure DB, Sato GH. Cholesterol requirement of NS-1 mouse myeloma cells for growth in serum-free medium. *Mol Biol Med*. 1984;2(2):121-134.
4. Gorfien S, Paul B, Walowitz J, Keem R, Biddle W, Jayme D. Growth of NS0 cells in protein-free, chemically defined medium. *Biotechnol Prog*. 2000;16(5):682-687.
5. Savonnière S, Zeghari N, Miccoli L, Muller S, Maugras M, Donner M. Effects of lipid supplementation of culture media on cell growth, antibody production, membrane structure and dynamics in two hybridomas. *J Biotechnol*. 1996;48(1–2):161-173.
6. Hewlettet G, Duvinski M, Montalto J. Pentex ex-Cyte growth enhancement media supplement as a lipoprotein additive for mammalian cell culture. *Miles Sci*. 1989;9-14.
7. Brenneman DE, Spector AA. Utilization of ascites plasma very low density lipoprotein triglycerides by Ehrlich cells. *J Lipid Res*. 1974; 15(4):309-316.
8. de la Llera M, Rothblat G, Howard BV. Cell triacylglycerol accumulation from very low density lipoproteins isolated from normal and hypertriglyceridemic human sera. *Biochim Biophys Acta*. 1979;574(3): 414-422.
9. Howard BV, Howard WJ, De la Llera M, Kefalides NA. Triglyceride accumulation in cultured human fibroblasts: the effects of hypertriglyceridemic serum. *Atherosclerosis*. 1976;23(3):521-534.
10. Havel R. Lipoproteins and lipid transport. *Lipids Liporprot Drugs*. 1975; 63:37-59.
11. Behrens WA, Thompson JN, Madère R. Distribution of alpha-tocopherol in human plasma lipoproteins. *Am J Clin Nutr*. 1982;35(4): 691-696.
12. Boone C. A surveillance procedure applied to sera. In: Kruse P Jr, Patterson M Jr, eds. *Tissue Culture Methods and Applications*. Academic Press; 1973:677.
13. Spector AA, Mathur SN, Kaduce TL, Hyman BT. Lipid nutrition and metabolism of cultured mammalian cells. *Prog Lipid Res*. 1980;19(3–4):155-186.
14. Honn KV, Singley JA, Chavin W. Fetal bovine serum: a multivariate standard. *Proc Soc Exp Biol Med*. 1975;149(2):344-347.
15. Shah G. Why do we still use serum in the production of biopharmaceuticals? *Dev Biol Stand*. 1999;99:17-22.
16. Price PJ, Gregory EA. Relationship between in vitro growth promotion and biophysical and biochemical properties of the serum supplement. *In Vitro*. 1982;18(6):576-584.
17. Cnop M, Hannaert JC, Hoorens A, Eizirik DL, Pipeleers DG. Inverse relationship between cytotoxicity of free fatty acids in pancreatic islet cells and cellular triglyceride accumulation. *Diabetes*. 2001;50(8): 1771-1777.
18. Ontko JA. Physical and chemical changes in isolated chylomicrons: prevention by EDTA. *J Lipid Res*. 1970;11(4):367-375.
19. Gerry AB, Satchell L, Leake DS. A novel method for production of lipid hydroperoxide- or oxysterol-rich low-density lipoprotein. *Atherosclerosis*. 2008;197(2):579-587.
20. Chang YH, Abdalla DS, Sevanian A. Characterization of cholesterol oxidation products formed by oxidative modification of low density lipoprotein. *Free Radic Biol Med*. 1997;23(2):202-214.
21. Jira W, Spiteller G, Carson W, Schramm A. Strong increase in hydroxy fatty acids derived from linoleic acid in human low density lipoproteins of atherosclerotic patients. *Chem Phys Lipids*. 1998;91(1):1-11.
22. Kitano S, Yoshida Y, Kawano K, Hibi N, Niki E. Oxidative status of human low density lipoprotein isolated by anion-exchange high-performance liquid chromatography—assessment by total hydroxyoctadecadienoic acid, 7-hydroxycholesterol, and 8-iso-prostaglandin F(2alpha). *Anal Chim Acta*. 2007;585(1):86-93.
23. Mori TA, Croft KD, Puddey IB, Beilin LJ. Analysis of native and oxidized low-density lipoprotein oxysterols using gas chromatography–mass spectrometry with selective ion monitoring. *Redox Rep*. 1996; 2(1):25-34.
24. Björkerud B, Björkerud S. Contrary effects of lightly and strongly oxidized LDL with potent promotion of growth versus apoptosis on arterial smooth muscle cells, macrophages, and fibroblasts. *Arterioscler Thromb Vasc Biol*. 1996;16(3):416-424.
25. Björkerud S, Björkerud B. Apoptosis is abundant in human atherosclerotic lesions, especially in inflammatory cells (macrophages and T cells), and may contribute to the accumulation of gruel and plaque instability. *Am J Pathol*. 1996;149(2):367-380.
26. Harada-Shiba M, Kinoshita M, Kamido H, Shimokado K. Oxidized low density lipoprotein induces apoptosis in cultured human umbilical vein endothelial cells by common and unique mechanisms. *J Biol Chem*. 1998;273(16):9681-9687.
27. Escargueil-Blanc I, Salvayre R, Nègre-Salvayre A. Necrosis and apoptosis induced by oxidized low density lipoproteins occur through two calcium-dependent pathways in lymphoblastoid cells. *FASEB J*. 1994;8(13):1075-1080.
28. Escargueil-Blanc I, Meilhac O, Pieraggi MT, Arnal JF, Salvayre R, Nègre-Salvayre A. Oxidized LDLs induce massive apoptosis of cultured human endothelial cells through a calcium-dependent pathway. Prevention by aurintricarboxylic acid. *Arterioscler Thromb Vasc Biol*. 1997;17(2):331-339.
29. Rusiñol AE, Yang L, Thewke D, Panini SR, Kramer MF, Sinensky MS. Isolation of a somatic cell mutant resistant to the induction of apoptosis by oxidized low density lipoprotein. *J Biol Chem*. 2000;275(10): 7296-7303.
30. Sugawa M, Ikeda S, Kushima Y, Takashima Y, Cynshi O. Oxidized low density lipoprotein caused CNS neuron cell death. *Brain Res*. 1997; 761(1):165-172.
31. Hughes H, Mathews B, Lenz ML, Guyton JR. Cytotoxicity of oxidized LDL to porcine aortic smooth muscle cells is associated with the oxysterols 7-ketocholesterol and 7-hydroxycholesterol. *Arterioscler Thromb*. 1994;14(7):1177-1185.
32. Draczynska-Lusiak B, Chen YM, Sun AY. Oxidized lipoproteins activate NF-kappaB binding activity and apoptosis in PC12 cells. *Neuroreport*. 1998;9(3):527-532.
33. Márta Z, Bobály B, Fekete J, et al. Simultaneous determination of thirteen different steroid hormones using micro UHPLC–MS/MS with on-line SPE system. *J Pharm Biomed Anal*. 2018;150:258-267.
34. Baraibar MA, Ladouce R, Friguet B. Proteomic quantification and identification of carbonylated proteins upon oxidative stress and during cellular aging. *J Proteome*. 2013;92:63-70.
35. Hessler JR, Morel DW, Lewis LJ, Chisolm GM. Lipoprotein oxidation and lipoprotein-induced cytotoxicity. *Arteriosclerosis*. 1983;3(3):215-222.
36. van den Berg R, Haenen G, van den Berg H, Bast A. Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem*. 1999;66(4):511-517.
37. Lucio M, Nunes C, Gaspar D, Ferreira H, Lima J, Reis S. Antioxidant activity of vitamin E and Trolox: understanding of the factors that govern lipid peroxidation studies In vitro. *Food Biophys*. 2009;4: 312-320.
38. Forrest V, Kang Y, McClain D, Robinson D, Ramakrishnan N. Oxidative stress-induced apoptosis prevented by trolox. *Free Radic Biol Med*. 1994;16:675-684.
39. Hauck AK, Bernlohr DA. Oxidative stress and lipotoxicity. *J Lipid Res*. 2016;57(11):1976-1986.
40. Sato JD, Cao HT, Kayada Y, et al. Effects of proximate cholesterol precursors and steroid hormones on mouse myeloma growth in

serum-free medium. *In Vitro Cell Dev Biol*. 1988;24(12):1223-1228.
41. Yang LL, Chen H, Wang J, et al. 4-HNE induces apoptosis of human retinal pigment epithelial cells by modifying HSP70. *Curr Med Sci*. 2019;39(3):442-448.
42. Yin G, Wang Y, Cen XM, Yang M, Liang Y, Xie QB. Lipid peroxidation-mediated inflammation promotes cell apoptosis through activation of NF-κB pathway in rheumatoid arthritis synovial cells. *Mediat Inflamm*. 2015;2015:460310.
43. Christ M, Luu B, Mejia JE, Moosbrugger I, Bischoff P. Apoptosis induced by oxysterols in murine lymphoma cells and in normal thymocytes. *Immunology*. 1993;78(3):455-460.
44. Bakos JT, Johnson BH, Thompson EB. Oxysterol-induced cell death in human leukemic T-cells correlates with oxysterol binding protein occupancy and is independent of glucocorticoid-induced apoptosis. *J Steroid Biochem Mol Biol*. 1993;46(4):415-426.
45. Aupeix K, Weltin D, Mejia JE, et al. Oxysterol-induced apoptosis in human monocytic cell lines. *Immunobiology*. 1995;194(4–5):415-428.
46. Nishio E, Arimura S, Watanabe Y. Oxidized LDL induces apoptosis in cultured smooth muscle cells: a possible role for 7-ketocholesterol. *Biochem Biophys Res Commun*. 1996;223(2):413-418.
47. Zhang J, Xue Y, Jondal M, Sjövall J. 7alpha-hydroxylation and 3-dehydrogenation abolish the ability of 25-hydroxycholesterol and 27-hydroxycholesterol to induce apoptosis in thymocytes. *Eur J Biochem*. 1997;247(1):129-135.
48. Horton AA, Fairhurst S. Lipid peroxidation and mechanisms of toxicity. *Crit Rev Toxicol*. 1987;18(1):27-79.
49. Girotti AW. Lipid hydroperoxide generation, turnover, and effector action in biological systems. *J Lipid Res*. 1998;39(8):1529-1542.
50. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science*. 1986;232(4746):34-47.

How to cite this article: Moisant R, Cowles E, Broutel L, et al. Characterization of lipoprotein supplement and influence of its oxidized lipid content on cell culture performance and monoclonal antibody production by a SP2/0 hybridoma cell line. *Biotechnol. Prog*. 2023;39(5):e3372. doi:10.1002/btpr.3372